

## AMENDMENTS TO THE CLAIMS

### In the claims:

Claims 1, 3, and 27-36 were the subject of the present Office Action. Claim 1 has been allowed. Please amend claims 27 and 32-35, cancel previously withdrawn claims 8-26 without prejudice, and add new claims 37-57, as shown in the following listing of claims, which will replace all prior versions and listings of claims in the application. Claims 8-26 were previously withdrawn from consideration as the result of a restriction requirement and are hereby canceled without prejudice to their pursuit in an appropriate continuation or divisional application.

### **Listing of claims:**

1. (previously presented) An isolated or purified enzyme exhibiting nicotianamine synthase activity, wherein the enzyme comprises the polypeptide having an amino acid sequence of SEQ ID NO: 1.

2. (canceled)

3. (previously presented) The enzyme according to claim 27, wherein the enzyme comprises the consensus amino acid sequence of <sub>199</sub>DVVFLAALVGM<sub>209</sub> (SEQ ID NO: 27).

4. – 26. (canceled)

27 (currently amended). An isolated or purified enzyme exhibiting nicotianamine synthase activity, wherein the enzyme:

a. is a polypeptide having at least 50% identity with an amino acid sequence of SEQ ID NO: 1, comprising at least one consensus sequence of SEQ ID NO: 1 that is:

(1) <sub>25</sub>LPXLSPSPXVDRLFTXLVXACVPXSPVDVTKL<sub>56</sub> (SEQ ID NO: 23)

(2) <sub>67</sub>LIRLCSXAEGXLEAHY<sub>82</sub> (SEQ ID NO: 24)

(3) <sub>92</sub>PLDHLGXFPY<sub>101</sub> (SEQ ID NO: 25)

(4) <sub>128</sub>VAFXGSGPLPFSS<sub>140</sub> (SEQ ID NO: 26)

(5) <sub>199</sub>DVVFLAALVGM<sub>209</sub> (SEQ ID NO: 27)

(6) <sub>253</sub>RGGFXVLAVXHP<sub>264</sub> (SEQ ID NO: 28); and

b. has more than 25% of the ~~relative~~ nicotianamine synthase activity of an equivalent amount of the ~~nicotianamine synthase~~ activity of the enzyme of SEQ ID NO: 1.

28 (previously presented). The enzyme of claim 27, wherein the polypeptide further comprises all of the conserved amino acid residues of SEQ ID NO: 1 that is:

L(11), K(14), I(15), I(22), L(25), L(28), P(30), L(37), F(38), L(41), V(42), C(45), P(47), D(52), V(53), Q(61), M(63), R(64), L(67), I(68), C(71), A(74), E(75), L(78), E(79), H(81), L(86), D(90), P(92), L(93), H(95), L(96), F(99), P(100), Y(101), N(104), Y(105), L(108), L(111), E(112), L(115), L(116), A(129), F(130), G(132), S(133), G(134), P(135), L(136), P(137), S(140), L(143), A(144), H(147), L(148), F(153), N(155), A(162), N(163), A(166), L(169), R(180), M(181), F(183), T(185), L(195), D(199), V(200), V(201), F(202), L(203), A(204), A(205), V(207), G(208), M(209), K(214), H(220), L(221), H(224), M(225), G(228), A(229), L(231), R(239), F(241), L(242), Y(243), P(244), V(246), G(255), F(256), V(258), L(259), V(261), H(263), P(264), V(268), N(270), S(271), K(277) (SEQ ID NO: 29).

29 (previously presented). The enzyme of claim 27, wherein the polypeptide has more than 90% identity with an amino acid sequence of SEQ ID NO: 1.

30 (previously presented). The enzyme of claim 27, wherein the polypeptide has more than 95% identity with an amino acid sequence of SEQ ID NO: 1.

31 (previously presented). The enzyme of claim 27, wherein the nicotianamine synthase activity is measured in an assay in a comparison with the enzyme of SEQ ID NO:1.

32 (previously presented). The enzyme of claim 27, wherein the enzyme:

- i. is isolated or purified from a plant; or
- ii. is expressed directly or indirectly from a nucleic acid isolated or purified from a plant; or
- iii. is expressed directly or indirectly from a chimeric nucleic acid at least partially isolated or purified from a plant.

33 (previously presented) The enzyme of claim ~~[[32,]]~~ 27, wherein the enzyme is isolated or purified from barley.

34. (previously presented) The enzyme of claim ~~[[32,]]~~ 27, wherein said enzyme is isolated or purified from *Oryza sativa*.

35 (currently amended). A mutated enzyme exhibiting nicotianamine synthase activity, wherein the enzyme:

- a. is a polypeptide having more than ~~[[90%]]~~ 95% identity with an amino acid sequence of SEQ ID NO: 1, comprising at least one consensus sequence of SEQ ID NO: 1 that is:

(1) <sub>25</sub>LPXLSPSPXVDRLFTXLVXACVPXSPVDVTKL<sub>56</sub> (SEQ ID NO: 23)

(2) <sub>67</sub>LIRLCSXAEGXLEAHY<sub>82</sub> (SEQ ID NO: 24)

- (3) <sup>92</sup>PLDHLGXFPY<sub>101</sub> (SEQ ID NO: 25)
- (4) <sup>128</sup>VAFXGSGPLPFSS<sub>140</sub> (SEQ ID NO: 26)
- (5) <sup>199</sup>DVVFLAALVGM<sub>209</sub> (SEQ ID NO: 27)
- (6) <sup>253</sup>RGGFVVLAVXHP<sub>264</sub> (SEQ ID NO: 28); and

- b. has more than 25% of the ~~relative~~ nicotianamine synthase activity of an equivalent amount of the ~~nicotianamine synthase activity of the enzyme of SEQ ID NO:1.~~

36 (previously presented). The enzyme of claim 35, wherein the polypeptide further comprises all of the conserved amino acid residues of SEQ ID NO: 1 that is:

L(11), K(14), I(15), I(22), L(25), L(28), P(30), L(37), F(38), L(41), V(42), C(45), P(47), D(52), V(53), Q(61), M(63), R(64), L(67), I(68), C(71), A(74), E(75), L(78), E(79), H(81), L(86), D(90), P(92), L(93), H(95), L(96), F(99), P(100), Y(101), N(104), Y(105), L(108), L(111), E(112), L(115), L(116), A(129), F(130), G(132), S(133), G(134), P(135), L(136), P(137), S(140), L(143), A(144), H(147), L(148), F(153), N(155), A(162), N(163), A(166), L(169), R(180), M(181), F(183), T(185), L(195), D(199), V(200), V(201), F(202), L(203), A(204), A(205), V(207), G(208), M(209), K(214), H(220), L(221), H(224), M(225), G(228), A(229), L(231), R(239), F(241), L(242), Y(243), P(244), V(246), G(255), F(256), V(258), L(259), V(261), H(263), P(264), V(268), N(270), S(271), K(277) (SEQ ID NO: 29).

37 (new). The enzyme of claim 35, wherein the nicotianamine synthase activity is measured in an assay in a comparison with the enzyme of SEQ ID NO:1.

38 (new). The enzyme of claim 35, wherein the polypeptide has more than 97% identity with an amino acid sequence of SEQ ID NO: 1.

39 (new). The enzyme of claim 27, wherein the polypeptide has more than 97% identity with an amino acid sequence of SEQ ID NO: 1.

40 (new). The enzyme of claim 27, wherein the enzyme is isolated or purified from a plant, either as a polypeptide or as a nucleic acid, which is used to express a polypeptide.

41 (new). An isolated, purified, or mutated enzyme exhibiting nicotianamine synthase activity; wherein the enzyme comprises an active fragment of an amino acid sequence of SEQ ID NO: 1, the active fragment comprising a polypeptide, wherein the polypeptide:

- a. comprises at least one consensus sequence of SEQ ID NO: 1 that is:
  - (1) <sub>25</sub>LPXLSPSPXVDRLFTXLVXACVPXSPVDVTKL<sub>56</sub> (SEQ ID NO: 23)
  - (2) <sub>67</sub>LIRLCSXAEGXLEAHY<sub>82</sub> (SEQ ID NO: 24)
  - (3) <sub>92</sub>PLDHLGXFPY<sub>101</sub> (SEQ ID NO: 25)
  - (4) <sub>128</sub>VAFXGSGPLPFSS<sub>140</sub> (SEQ ID NO: 26)
  - (5) <sub>199</sub>DVVFLAALVGM<sub>209</sub> (SEQ ID NO: 27)
  - (6) <sub>253</sub>RGGFXVLAVXHP<sub>264</sub> (SEQ ID NO: 28); and
- b. has more than 25% of the relative nicotianamine synthase activity of the enzyme of SEQ ID NO:1.

42 (new). The enzyme of claim 27 or claim 41, wherein said enzyme is isolated or purified from *Arabidopsis thaliana*.

43 (new). An isolated or purified barley enzyme exhibiting nicotianamine synthase activity, wherein:

- a. the enzyme is:
  - i. isolated or purified from barley; or

- ii. expressed directly or indirectly from a nucleic acid isolated or purified from barley; or
- iii. expressed directly or indirectly from a chimeric nucleic acid at least partially isolated or purified from barley;
- b. the enzyme comprises a polypeptide having at least 50% identity with an amino acid sequence of SEQ ID NO: 1, comprising at least one consensus sequence of SEQ ID NO: 1 that is:
  - (1) <sub>25</sub>LPXLSPSPXVDRLFTXLVXACVPXSPVDVTKL<sub>56</sub> (SEQ ID NO: 23)
  - (2) <sub>67</sub>LIRLC SXAEGXLEAHY<sub>82</sub> (SEQ ID NO: 24)
  - (3) <sub>92</sub>PLDHLGXFPY<sub>101</sub> (SEQ ID NO: 25)
  - (4) <sub>128</sub>VAFXGSGPLPFSS<sub>140</sub> (SEQ ID NO: 26)
  - (5) <sub>199</sub>DVVFLAALVGM<sub>209</sub> (SEQ ID NO: 27)
  - (6) <sub>253</sub>RGGFXVLAVXHP<sub>264</sub> (SEQ ID NO: 28); and
- c. the enzyme has more than 25% of the relative nicotianamine synthase activity of the enzyme of SEQ ID NO:1.

44 (new). The enzyme of claim 43, wherein the polypeptide further comprises all of the conserved amino acid residues of SEQ ID NO: 1 that is:

L(11), K(14), I(15), I(22), L(25), L(28), P(30), L(37), F(38), L(41), V(42), C(45), P(47), D(52), V(53), Q(61), M(63), R(64), L(67), I(68), C(71), A(74), E(75), L(78), E(79), H(81), L(86), D(90), P(92), L(93), H(95), L(96), F(99), P(100), Y(101), N(104), Y(105), L(108), L(111), E(112), L(115), L(116), A(129), F(130), G(132), S(133), G(134), P(135), L(136), P(137), S(140), L(143), A(144), H(147), L(148), F(153), N(155), A(162), N(163), A(166), L(169), R(180), M(181), F(183), T(185), L(195), D(199), V(200), V(201), F(202), L(203), A(204), A(205), V(207), G(208), M(209), K(214), H(220), L(221), H(224), M(225), G(228), A(229), L(231), R(239),

F(241), L(242), Y(243), P(244), V(246), G(255), F(256), V(258), L(259), V(261),  
H(263), P(264), V(268), N(270), S(271), K(277) (SEQ ID NO: 29).

45 (new). The enzyme of claim 43, wherein the polypeptide has more than 90% identity with an amino acid sequence of SEQ ID NO: 1.

46 (new). The enzyme of claim 43, wherein the polypeptide has more than 95% identity with an amino acid sequence of SEQ ID NO: 1.

47 (new). The enzyme of claim 43, wherein the nicotianamine synthase activity is measured in an assay in a comparison with the enzyme of SEQ ID NO: 1.

48 (new). An isolated or purified enzyme exhibiting nicotianamine synthase activity, wherein:

- a. the enzyme is:
  - i. isolated or purified from rice; or
  - ii. expressed directly or indirectly from a nucleic acid isolated or purified from rice; or
  - iii. expressed directly or indirectly from a chimeric nucleic acid at least partially isolated or purified from rice;
- b. the enzyme comprises a polypeptide having at least 50% identity with an amino acid sequence of SEQ ID NO: 1, comprising at least one consensus sequence of SEQ ID NO: 1 that is:

(1) <sub>25</sub>LPXLSPSPXVDRLFTXLVXACVPXSPVDVTKL<sub>56</sub> (SEQ ID NO: 23)

(2) <sub>67</sub>LIRLCSXAEGXLEAHY<sub>82</sub> (SEQ ID NO: 24)

- (3) <sub>92</sub>PLDHLGXFPY<sub>101</sub> (SEQ ID NO: 25)
- (4) <sub>128</sub>VAFXGSGPLPFSS<sub>140</sub> (SEQ ID NO: 26)
- (5) <sub>199</sub>DVVFLAALVGM<sub>209</sub> (SEQ ID NO: 27)
- (6) <sub>253</sub>RGGFXVLAVXHP<sub>264</sub> (SEQ ID NO: 28); and

c. the enzyme has more than 25% of the relative nicotianamine synthase activity of the enzyme of SEQ ID NO:1.

49 (new). The enzyme of claim 48, wherein the polypeptide further comprises all of the conserved amino acid residues of SEQ ID NO: 1 that is:

L(11), K(14), I(15), I(22), L(25), L(28), P(30), L(37), F(38), L(41), V(42), C(45), P(47), D(52), V(53), Q(61), M(63), R(64), L(67), I(68), C(71), A(74), E(75), L(78), E(79), H(81), L(86), D(90), P(92), L(93), H(95), L(96), F(99), P(100), Y(101), N(104), Y(105), L(108), L(111), E(112), L(115), L(116), A(129), F(130), G(132), S(133), G(134), P(135), L(136), P(137), S(140), L(143), A(144), H(147), L(148), F(153), N(155), A(162), N(163), A(166), L(169), R(180), M(181), F(183), T(185), L(195), D(199), V(200), V(201), F(202), L(203), A(204), A(205), V(207), G(208), M(209), K(214), H(220), L(221), H(224), M(225), G(228), A(229), L(231), R(239), F(241), L(242), Y(243), P(244), V(246), G(255), F(256), V(258), L(259), V(261), H(263), P(264), V(268), N(270), S(271), K(277) (SEQ ID NO: 29).

50 (new). The enzyme of claim 48, wherein the polypeptide has more than 90% identity with an amino acid sequence of SEQ ID NO: 1.

51 (new). The enzyme of claim 48, wherein the polypeptide has more than 95% identity with an amino acid sequence of SEQ ID NO: 1.



52 (new). The enzyme of claim 48, wherein the nicotianamine synthase activity is measured in an assay in a comparison with the enzyme of SEQ ID NO:1.

53 (new). An isolated or purified enzyme exhibiting nicotianamine synthase activity, wherein:

- a. the enzyme is:
  - i. isolated or purified from *Arabidopsis thaliana*; or
  - ii. expressed directly or indirectly from a nucleic acid isolated or purified from *Arabidopsis thaliana*; or
  - iii. expressed directly or indirectly from a chimeric nucleic acid at least partially isolated or purified from *Arabidopsis thaliana*;
- b. the enzyme comprises a polypeptide having at least 50% identity with an amino acid sequence of SEQ ID NO: 1, comprising at least one consensus sequence of SEQ ID NO: 1 that is:
  - (1) <sub>25</sub>LPXLSPSPXVDRLFTXLVXACVPXSPVDVTKL<sub>56</sub> (SEQ ID NO: 23)
  - (2) <sub>67</sub>LIRLCSXAEGXLEAHY<sub>82</sub> (SEQ ID NO: 24)
  - (3) <sub>92</sub>PLDHLGXFPY<sub>101</sub> (SEQ ID NO: 25)
  - (4) <sub>128</sub>VAFXGSGPLPFSS<sub>140</sub> (SEQ ID NO: 26)
  - (5) <sub>199</sub>DVVFLAALVGM<sub>209</sub> (SEQ ID NO: 27)
  - (6) <sub>253</sub>RGGFXVLAVXHP<sub>264</sub> (SEQ ID NO: 28); and
- c. the enzyme has more than 25% of the relative nicotianamine synthase activity of the enzyme of SEQ ID NO:1.

54 (new). The enzyme of claim 53, wherein the polypeptide further comprises all of the conserved amino acid residues of SEQ ID NO: 1 that is:

L(11), K(14), I(15), I(22), L(25), L(28), P(30), L(37), F(38), L(41), V(42), C(45), P(47), D(52), V(53), Q(61), M(63), R(64), L(67), I(68), C(71), A(74), E(75), L(78), E(79), H(81), L(86), D(90), P(92), L(93), H(95), L(96), F(99), P(100), Y(101), N(104), Y(105), L(108), L(111), E(112), L(115), L(116), A(129), F(130), G(132), S(133), G(134), P(135), L(136), P(137), S(140), L(143), A(144), H(147), L(148), F(153), N(155), A(162), N(163), A(166), L(169), R(180), M(181), F(183), T(185), L(195), D(199), V(200), V(201), F(202), L(203), A(204), A(205), V(207), G(208), M(209), K(214), H(220), L(221), H(224), M(225), G(228), A(229), L(231), R(239), F(241), L(242), Y(243), P(244), V(246), G(255), F(256), V(258), L(259), V(261), H(263), P(264), V(268), N(270), S(271), K(277) (SEQ ID NO: 29).

55 (new). The enzyme of claim 53, wherein the polypeptide has more than 90% identity with an amino acid sequence of SEQ ID NO: 1.

56 (new). The enzyme of claim 53, wherein the polypeptide has more than 95% identity with an amino acid sequence of SEQ ID NO: 1.

57 (new). The enzyme of claim 53, wherein the nicotianamine synthase activity is measured in an assay in a comparison with the enzyme of SEQ ID NO:1.